Ultrastructure of the Rat Pancreas After Experimental Duct Ligation

I. The Role of Apoptosis and Intraepithelial Macrophages in Acinar Cell Deletion

N. I. WALKER

From the Department of Pathology, University of Queensland, Brisbane, Australia

After ligation of the rat pancreas, acinar cells disappeared from the distal gland within 5 days. Necrosis was responsible for minor cell loss during the first 24 hours, but most of the acinar cells were deleted by apoptosis. This distinctive form of cell death, which has been implicated in atrophy of other tissues, was characterized by cellular condensation followed by surface budding to produce membrane-bounded apoptotic bodies. Most of these were ingested and degraded by mononuclear phagocytes resident within the epithelium and a few by adjoining acinar cells. As atrophy progressed, the phagocytes increased in number through division and immigration of monocytes. The apoptotic deletion of acinar cells was characteristically effected without basement membrane disruption and was accompanied by simultaneous duct cell proliferation. The consequent rapid glandular remodeling resulted in reduction of the lobules to clusters of ductules in a collagenous stroma. (Am J Pathol 1987, 126:439–451)

IN THE ATROPHY of the mammalian pancreas that follows duct obstruction, islet tissue is preserved, but acinar cells progressively disappear, and the exocrine glandular lobules are reduced to groups of small ductules within a fibrous stroma.[1-4] In man, such changes occur after duct occlusion by gallstones or carcinoma and in chronic calcific pancreatitis and cystic fibrosis.[5] Ultrastructural studies designed to elucidate the cellular mechanisms involved in the evolution of these lesions in experimental animals have stressed the role of autophagy in removing acinar cell organelles and in reducing the size of these cells,[2,4] and it has been suggested that many of the cells lining the ductules seen in the advanced stages of atrophy are dedifferentiated acinar cells.[2,6,7] Little attention has been paid to the possibility that deletion of acinar cells is involved in the evolution of atrophy; where its significance has been appreciated, it has been considered to be a result of necrosis[8,9] or of a combination of lytic cell death[4,10] and wasting cell death consequent on extreme autophagy.[4]

Previous light-microscopic studies in this laboratory have shown that extensive acinar cell death and duct cell proliferation are both involved in the genesis of the lesion described above.[3] In the present study ultrastructural observations were recorded that identify the mode of cell death as apoptosis,[11-14] which is basically different from necrosis and which has been shown to be implicated in cell population kinetics in a variety of tissues. Most of the remnants of the dying cells were disposed of by macrophages normally resident within the epithelium; these cells have previously received little attention.

Materials and Methods

Randomly bred male Sprague–Dawley rats weighing between 200 and 300 g were used. They were fed a pellet diet containing 22% protein, 4–6% fat, and

Supported by Research Funds from the University of Queensland.

Accepted for publication October 2, 1986.

Address reprint requests to N.I. Walker, Department of Pathology, Medical School, Herston 4006, Australia.

mineral and vitamin supplements. Control animals were not subjected to any experimental procedure. Experimental animals were anesthetized with pentobarbitone sodium given intraperitoneally, and partial ligation of the pancreas was performed through a left paramedian incision.[15] The lienal pancreas was freed from colonic mesentery, two silk ligatures (Ethicon K831, Ethnor Pty Ltd, Sydney, Australia) tied about the pancreas at the level of the superior dorsal pole of the spleen, and the parenchyma between the ligatures divided. The splenic vessels supplying the distal pancreas remained as a bridge between duodenal and lienal pancreas. Care was taken to avoid trauma to blood vessels and pancreas and to prevent drying of pancreatic tissue. The abdomen was then closed, the whole procedure taking approximately 10 minutes. More than 40% of pancreas by weight lay distal to the ligature[16]; it underwent regular and reproducible atrophy. Animals regained their preoperative weight within 2–3 days. Pancreatic tissue from two experimental animals was studied at each of the following intervals after operation: 1, 2, 3, 6, 12, 24, 36, 48, 60, 72, and 84 hours; 4, 5, 6, and 7 days; 2, 3, 4, 8, 12, 24, and 52 weeks.

To obtain tissue for histologic study and electron microscopy, animals were anesthetized and the pancreas perfused in sequence with 1) heparinized saline 2) 1% paraformaldehyde and 1.25% glutaraldehyde in cacodylate buffer, and 3) 4% paraformaldehyde and 5% glutaraldehyde in cacodylate buffer[17] via a catheter inserted retrogradely into the distal abdominal aorta. Pancreas proximal and distal to the ligature and from corresponding areas in control animals was removed. For light microscopy, 5-μ paraffin sections were prepared and stained with hematoxylin and eosin. For electron microscopy, tissue was diced and immersed in perfusate (3). The diced tissue was washed and then stored in cacodylate buffer. After postfixing in 1% osmium tetroxide for 1 hour, tissue was washed in distilled water, stained *en bloc* in 5% aqueous uranyl acetate, dehydrated through a series of graded alcohols, cleared in propylene oxide, and embedded in an Epon/Araldite mixture. Sections 1μ in thickness were cut on an LKB Ultratome V and stained with toluidine blue for use in selecting areas for detailed study. Ultrathin sections were picked up on uncoated copper grids, stained with lead citrate for 1–2 minutes, and examined in Hitachi H300 and JEOL S100 electron microscopes.

Results

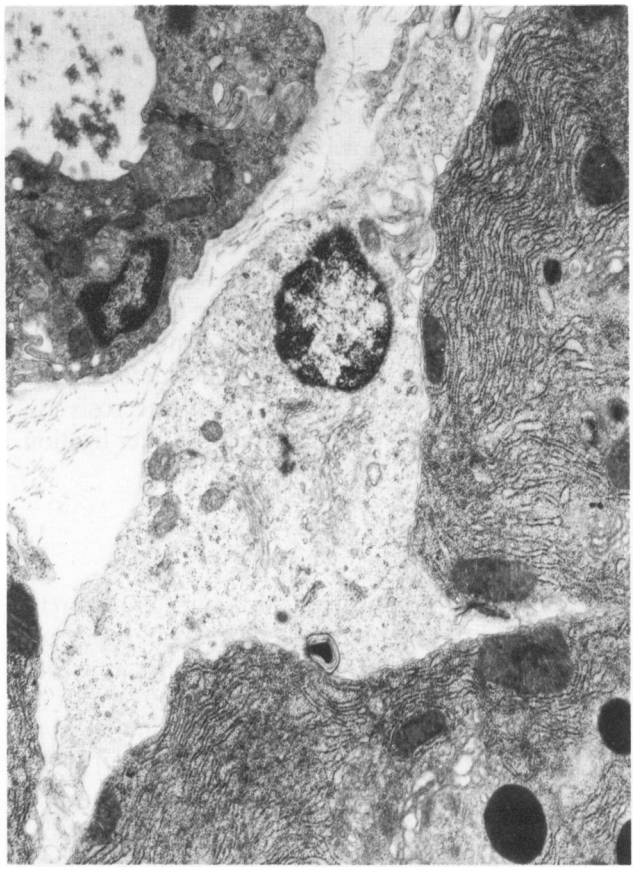
Normal Pancreas

Electron Microscopy

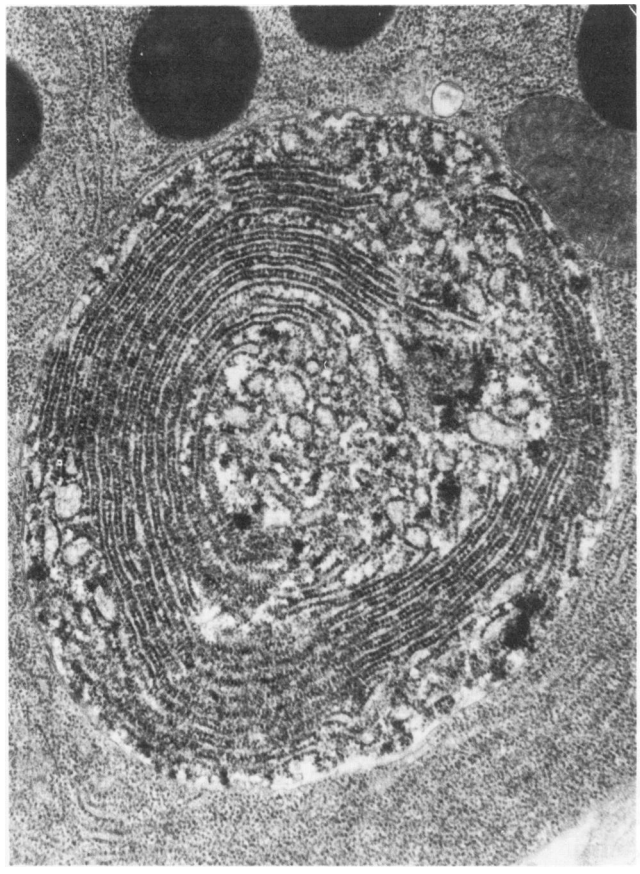
The ultrastructural appearance of the normal rat pancreas was, on the whole, in accord with previous descriptions.[18–20] However, several additional features were noted. First, occasional acinar cells showing the changes of cell death by apoptosis were found. Their appearance was similar to that observed in cells affected by this process in the duct-obstructed pancreas, and will be described in detail later. Second, cells with the characteristics of unstimulated mononuclear phagocytes were seen within the epithelial lining of acini. These cells exhibited marked phagocytic activity after ligation, playing a major part in removing cells undergoing apoptosis. They were basally situated between adjacent acinar cells (Figure 1) or between acinar and duct cells. Their nuclei were only infrequently included in the plane of section, and they often appeared as areas of membrane-bounded electron-lucent cytoplasm against the basement membrane. No more than one was seen per acinus. Similar cells were occasionally identified in the epithelium of intercalated and larger ducts. They were considerably outnumbered by interstitial mononuclear phagocytes in most sections.

The ultrastructure of the intraepithelial mononuclear phagocytes varied considerably. Smaller cells had rounded or slightly indented nuclei with abundant heterochromatin; showed small amounts of electron-lucent cytoplasm containing sparse mitochondria, strands of endoplasmic reticulum, and lysosomes; and bore short surface filopodia. Larger cells had large nuclei with broad indentations and less abundant heterochromatin marginated against the nuclear membrane, and showed copious electron-lucent cytoplasm containing a few small mitochondria, often multiple Golgi complexes, short strands of rough and smooth endoplasmic reticulum, residual bodies, pinocytotic vesicles, and lysosomes (Figure 1). The mononuclear phagocytes lacked junctional complexes with adjacent epithelial cells and had cell processes extending into the spaces between them; these spaces varied in width and often contained myelin figures. The cell processes were not seen passing through the basement membrane in normal animals.

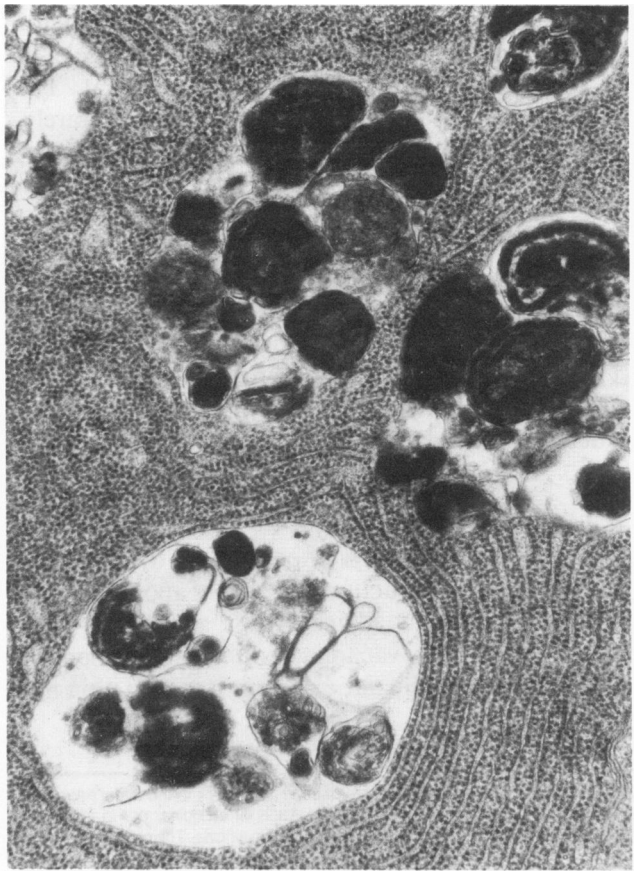
Figure 1 — Intraepithelial mononuclear phagocyte between acinar cells in normal pancreas. Note its basal position, electron-lucent cytoplasm, and absence of cell junctions. An intercalated duct lies nearby. (×11,700) **Figure 2** — Autophagic vacuole in an acinar cell in the distal pancreas 24 hours after ligation. (×22,000) **Figure 3** — Residual bodies in an acinar cell in the distal pancreas 24 hours after ligation. (×29,000) **Figure 4** — Necrosis of an acinar cell in the distal pancreas 1 hour after ligation. (×12,200)



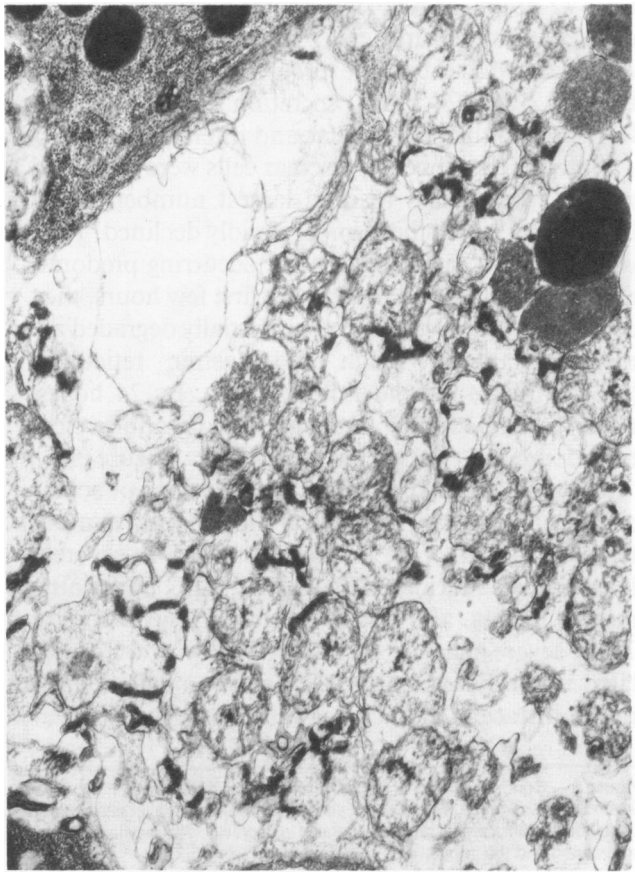
1



2



3



4

Pancreas After Ligation

Early Changes, Evidence of Injury, Necrosis

Light Microscopy

In the interstitium of the distal pancreas, increasing edema and infiltration by neutrophil leukocytes and mononuclear phagocytes occurred from 1 hour after ligation. Acinar lumens dilated and filled with secretion, and acinar cells partly degranulated; these changes progressed over the following 3 days. Cytoplasmic vacuolation of acinar and centroacinar cells of varying severity was seen in a patchy distribution during the first 24 hours, but later regressed. Occasional more severely vacuolated acinar cells with irregular clumping of nuclear chromatin and increased eosinophilia of their cytoplasm characteristic of necrosis were observed during the first 24 hours but not at later times.

Electron Microscopy

Within 2–3 hours of ligation, many zymogen granules in the adluminal cytoplasm of acinar cells were discharged into the acinar lumens. By 24 hours, the granules were conspicuously decreased in number and varied greatly in size; few remained by 72 hours. Acinar cell surface microvilli decreased in number over 72 hours, and myelin figures were found in increased numbers in acinar lumens from 2 hours. Acini and ducts became progressively dilated. Between 12 and 24 hours, secretion leaked from the lumens into the intercellular and interstitial spaces.

Autophagic vacuoles in acinar cells were increased within 2 hours and reached largest numbers at 24 hours, after which the number rapidly declined. They were found in most acinar cells, occurring predominantly in their apical half. In the first few hours, they contained structurally intact or partially degraded zymogen granules, rough endoplasmic reticulum (RER) (Figure 2), and mitochondria. By 24 hours, many contained only amorphous and granular electron-dense material and myelin figures (Figure 3).

Within 2 hours of ligation, small groups of acinar cells in an irregular distribution showed mild to moderate dilatation of RER. Occasional more severely affected cells had markedly dilated RER containing myelin figures, and abundant autophagic vacuoles. These severe changes were associated with widely dilated intercellular spaces; the development of large, round intracellular vacuoles extending from the nucleus to the lumen and indenting the nucleus; and the presence of neutrophils and mononuclear phagocytes between the epithelial cells.

Necrosis of acinar cells was rare, occurring only in the vicinity of cells showing other evidence of severe injury and only in the first 24 hours after ligation. Necrotic cells showed severe swelling, granular and amorphous densities in mitochondria, and rupture of mitochondrial and cell membranes (Figure 4). Because the number of necrotic acinar cells was small and because they were found at a time when a decrease in acinar cell numbers was not detectable by light microscopy, it is most unlikely that necrosis contributed significantly to acinar cell loss after ligation.

Acinar Cell Deletion by Apoptosis

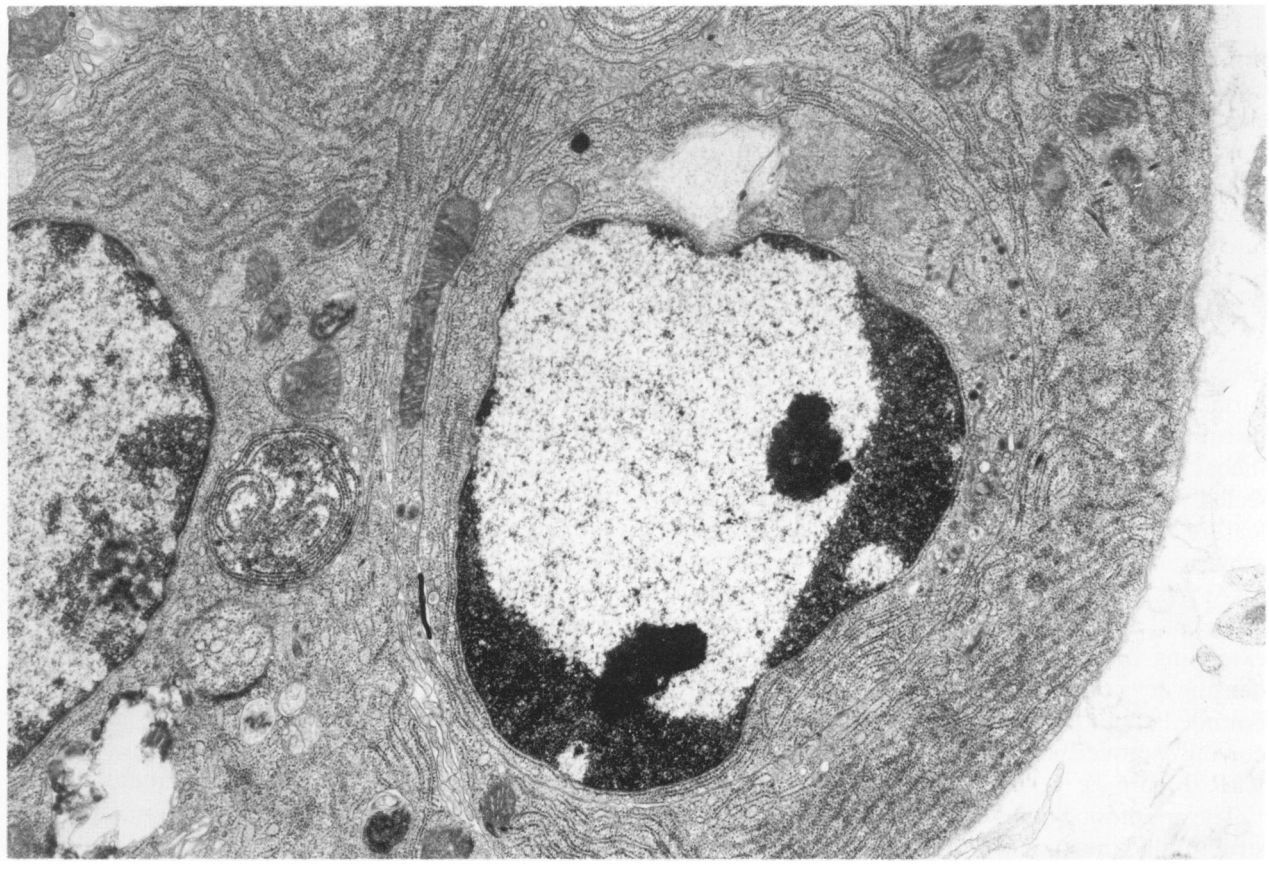
Light Microscopy

Twenty-four hours after ligation, the lobular architecture was little altered, and a decrease in acinar cell numbers was barely detectable. By 5 days, however, no recognizable acinar cells remained. In the interim, abundant evidence of acinar cell death by apoptosis[11-14] was seen by light microscopy. This included sharply marginated, often crescentic clumping of chromatin against the nuclear membrane; cell shrinkage; and cell and nuclear fragmentation forming rounded basophilic or eosinophilic cytoplasmic globules (apoptotic bodies)[11-14] in which specks of pyknotic chromatin were frequently present. The apoptotic bodies lay within the epithelium or, rarely, appeared in duct lumens. Acinar cell death by apoptosis increased from 24 hours, reached a peak at 3 days, and fell to low but still increased levels by 5 days. Usually only one cell per acinus showed these changes at any one time, but occasionally two or three acinar cells were involved simultaneously. Apoptosis of other cell types was not recognized during this period. The most rapid reduction in acinar cell numbers, estimated by semiquantitative analysis of sections, coincided with the peak incidence of apoptosis at 3 days.

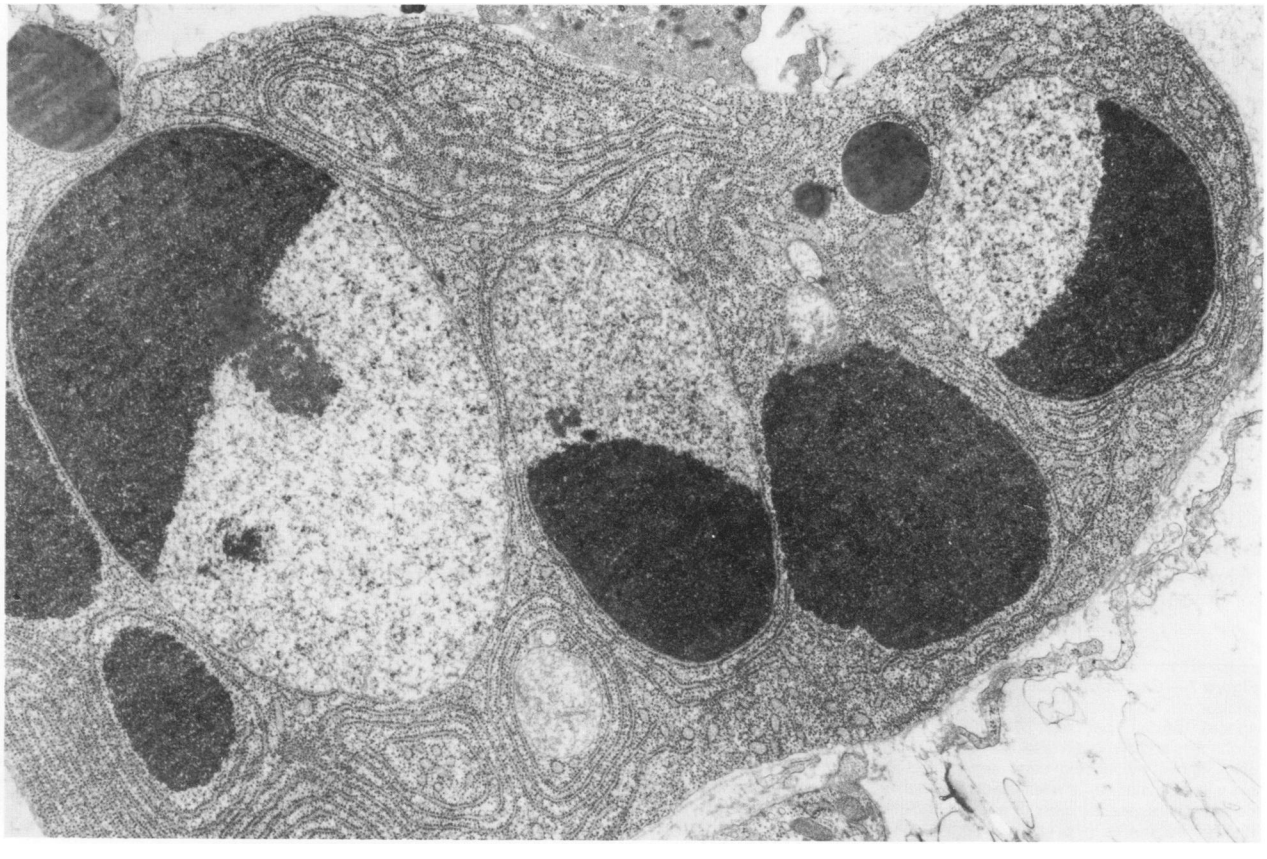
Electron Microscopy

Between 1 and 5 days after ligation, dying acinar cells showed ultrastructural changes of apoptosis.[11-14] The apoptotic and adjacent cells sometimes contained autophagic vacuoles, but large numbers of such vacuoles and dilatation of ergastoplasm were not seen at this stage.

Figure 5 — Condensation of chromatin and convolution of the membrane of an acinar cell nucleus, changes indicative of early apoptosis, in the distal pancreas 60 hours after ligation. Autophagolysosomes are seen in adjacent acinar cell cytoplasm. (×13,500) **Figure 6** — Apoptosis of an acinar cell in the distal pancreas 24 hours after ligation. It contains multiple nuclear fragments with marginated condensed chromatin and RER arranged in whorls. The cellular outline is convoluted and the intercellular space enlarged. (×13,500)



5



6

Nuclei in some of the cells undergoing apoptosis showed condensation of chromatin against the nuclear membrane, the presence of rounded clumps of electron-dense granules, and convolution of the nuclear outline; there was a sharp line of demarcation between the condensed chromatin and the electron-lucent nuclear interior (Figure 5). In other cells, the nucleus had budded to form several, usually membrane-bounded nuclear fragments retaining the characteristic segregation of condensed chromatin (Figure 6). These nuclear changes were accompanied by condensation of cytoplasm and convolution of the cell surface (Figure 6), the latter being associated with disruption of junctional complexes with adjacent epithelial cells and creation of a space between the affected and adjacent cells (Figure 6). A conspicuous feature of early apoptosis in the acinar cells was rearrangement of the RER into a series of whorls (Figure 6). At this stage, occasional apoptotic cells took on an amoeboid appearance with pseudopodlike processes extending between adjacent acinar cells. The condensing cells eventually budded to form membrane-bounded apoptotic bodies, the cell cleavage often occurring between areas of concentrically arranged RER (Figure 7).

The apoptotic bodies contained closely packed but structurally intact RER and organelles, and some contained one or more nuclear fragments with characteristic nuclear chromatin segregation (Figures 7 and 8). Although a few apoptotic bodies lay between epithelial cells and the basement membrane, most had been phagocytosed by adjacent cells. They were rarely seen in acinar or duct lumens. In the first 24 hours, but not at later times, occasional larger bodies that had not been phagocytosed showed dilatation of the RER and the nuclear envelope and swelling of mitochondria. Degenerative change manifest as rupture of internal or external membranes was not seen prior to phagocytosis of apoptotic bodies.

A small proportion of apoptotic bodies appeared to be ingested by acinar cells (Figure 9) and even more rarely by duct epithelial cells. The majority, however, were ingested by intraepithelial mononuclear phagocytes (Figures 10–12). Apoptotic bodies within these cells could often be identified as being of acinar cell origin by their content of zymogen granules or abundant RER (Figures 10 and 12). Apoptotic bodies without nuclear material that had been phagocytosed by acinar cells could not be distinguished from autophagic vacuoles with certainty. Within the phagolysosomes of acinar cells and macrophages, progressive enzymatic degradation of the chromatin and organelles of the apoptotic bodies took place. Chromatin increased in density, the sharp demarcation between electron-dense and electron-lucent areas of the apoptotic nuclear fragments was lost, ribosomes became swollen and coarse, and membranes became indistinct (Figures 11 and 12). Multiple phagocytosed apoptotic bodies were commonly found in intraepithelial mononuclear phagocytes 60–72 hours after ligation (Figure 12), a period that corresponded with maximum acinar cell deletion. By 72 hours, many intraepithelial mononuclear phagocytes contained large residual bodies filled with granular and amorphous electron-dense material, lipid droplets, and myelin figures. Similar cells laden with residual bodies were not found in normal animals.

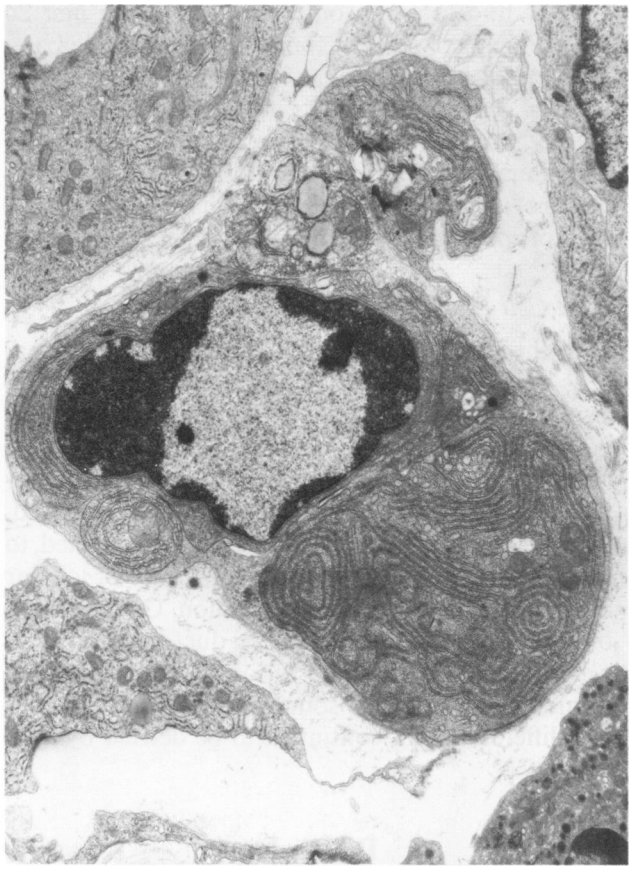
Evidence of acinar cell death by apoptosis was also rarely seen in control animals (Figure 13) and in the ligated pancreas within 24 hours of operation. In the normal pancreas, and in the ligated pancreas examined more than 24 hours after operation, apoptosis was the only form of acinar cell death detected. In all cases, early stages of apoptosis were found less frequently than the late degradative stages that occur after apoptotic bodies have been phagocytosed; this is a manifestation of the speed with which the early stages of apoptosis are effected.[12]

Changes in Intraepithelial Mononuclear Phagocytes

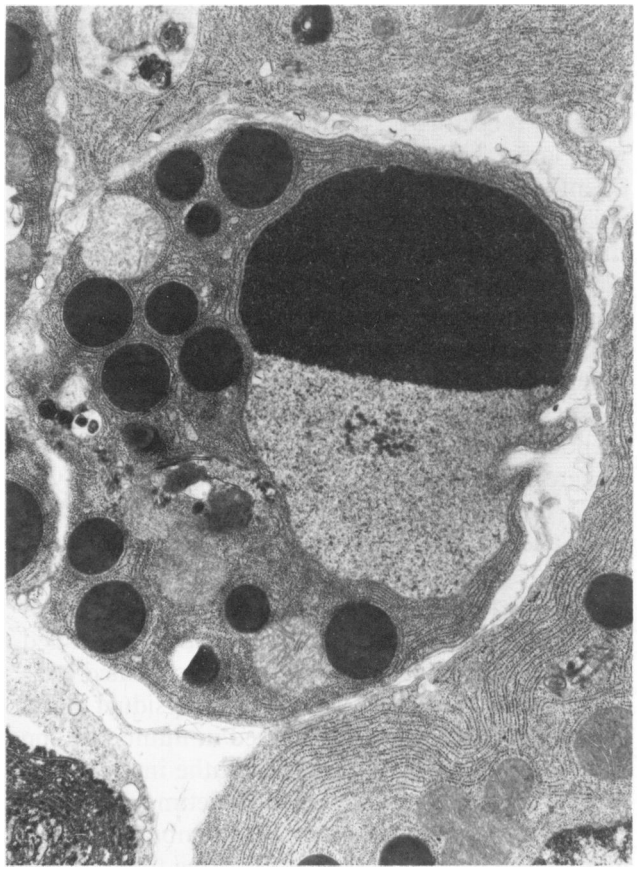
Light Microscopy

These cells were quite prominent during the phase of rapid apoptotic deletion of acinar epithelium; they could be recognized by their pale indented or folded nuclei and their abundant pale cytoplasm that contained phagocytosed apoptotic bodies. At other times, they were very inconspicuous; they could be detected in toluidine blue-stained 1-μ Epon sections but not in paraffin sections, where overlap of adjacent, more heavily stained cells prevented their visualization.

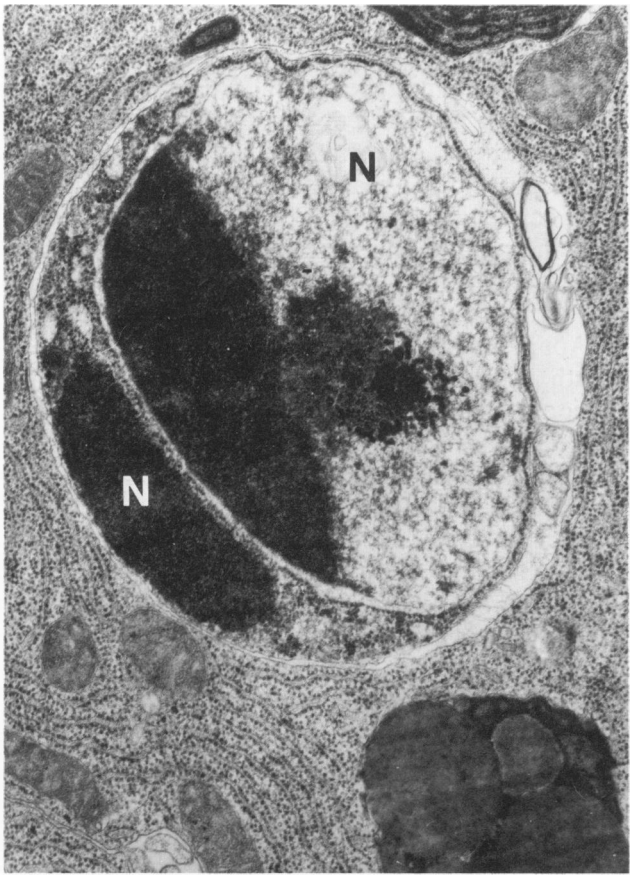
Figure 7—Cluster of apoptotic bodies in the distal pancreas 60 hours after ligation. They are surrounded by the cytoplasm of an intraepithelial mononuclear phagocyte. Note characteristic nuclear changes, condensed cytoplasm, and whorled RER. (×7300) **Figure 8**—Apoptotic body lying in the intercellular space in the distal pancreas 24 hours after ligation. It contains a large nuclear fragment with sharply segregated dense and lucent areas and tightly packed intact organelles and zymogen granules. (×9700) **Figure 9**—Probable apoptotic body containing two nuclear fragments (N) within a phagolysosome of an acinar cell. It shows evidence of early degradation. That the appearances might represent changes of apoptosis developing in the sequestered second nucleus of a binucleate acinar cell is a less likely alternative. Distal pancreas 24 hours after ligation. (×24,100) **Figure 10**—Engulfment of an apoptotic body by the cellular processes (*arrows*) of an intraepithelial mononuclear phagocyte in the distal pancreas 24 hours after ligation. The apoptotic body, which contains 3 nuclear fragments (*N*), can be identified as acinar cell in origin by its content of RER and zymogen granules. (×10,200)



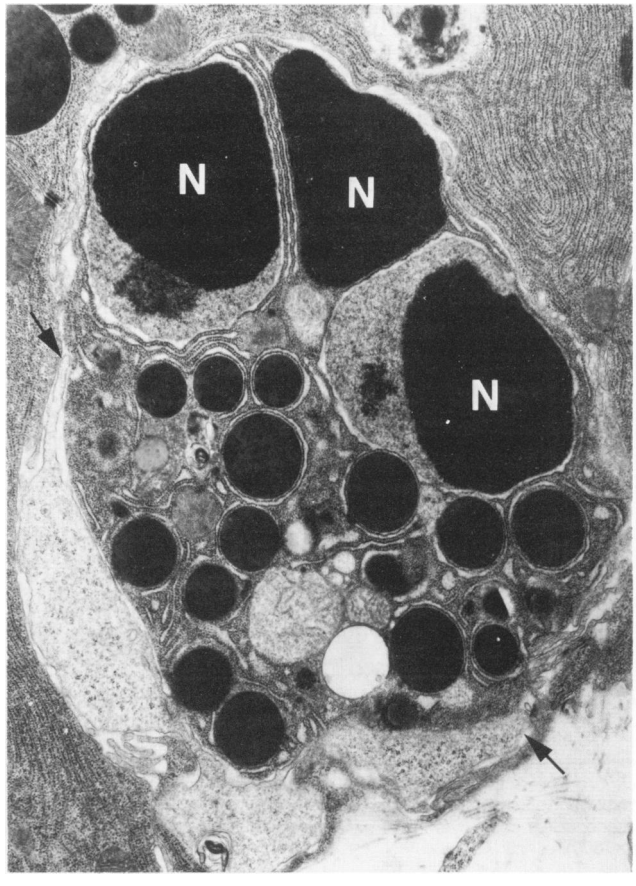
7



8



9



10

Electron Microscopy

After ligation, intraepithelial mononuclear phagocytes in the distal pancreas showed progressive changes reflecting activation. Within 6 hours, they were larger and therefore were included more frequently in ultrathin sections, and some showed phagocytotic activity. By 24 hours, they were larger still, their nuclei had less heterochromatin, and their cytoplasm contained greatly increased smooth endoplasmic reticulum, RER, Golgi complexes, mitochondria, glycogen, and lysosomes (Figure 14). Broad indentations of the nucleus on the side of the cell with most cytoplasm were frequent. Surface filopodia and pseudopodia were common, and phagocytic activity was greatly enhanced in many of the cells. On Days 2 and 3, the phagocytes showed even further increase in size, and their nuclei became larger and more rounded and had little heterochromatin and large nucleoli; phagosomes and residual bodies were common. The phagocytes now superficially resembled epithelial cells (Figure 11), but many contained large residual bodies. From 84 hours, such residual body-laden cells progressively decreased in number in the epithelium and began to appear in the interstitium. Moreover, their processes were sometimes observed extending through the basement membrane. In the interstitium, they shrank in size during the next few weeks, their nuclear heterochromatin increased, the number of organelles decreased, the residual bodies became smaller, and myelin figures were extruded into the extracellular space. After several months, the phagocytes were seen to be packed with amorphous material of varying electron density, lipid, and concentrically whorled membranous material (Figure 15). Over many months, their numbers decreased further, and they came to lie, along with increased numbers of mast cells, about large vessels and ducts. They could be detected by light microscopy by their lipofuscin content.

In addition to increasing in size, the intraepithelial mononuclear phagocytes also increased in number in the obstructed pancreas between 2 and 4 days after ligation, and groups of two to three were seen within the remaining epithelium of acini at this time. Some were observed undergoing mitosis between 2 and 14 days (Figure 16). In addition, cells with the morphologic characteristics of lymphocytes and monocytes were found in increased numbers in the pancreatic epithelium in the first days after duct ligation and in smaller numbers for some months. In the first 24 hours, neutrophils were found adjacent to the basement membrane within the epithelium, most often in areas of acinar cell necrosis. A single neutrophil was seen adjacent to an apoptotic body, the neutrophil being separated from the apoptotic body by the processes of an intraepithelial macrophage. No phagocytosis of apoptotic bodies by neutrophils was detected.

Duct Cell Proliferation and Differentiation

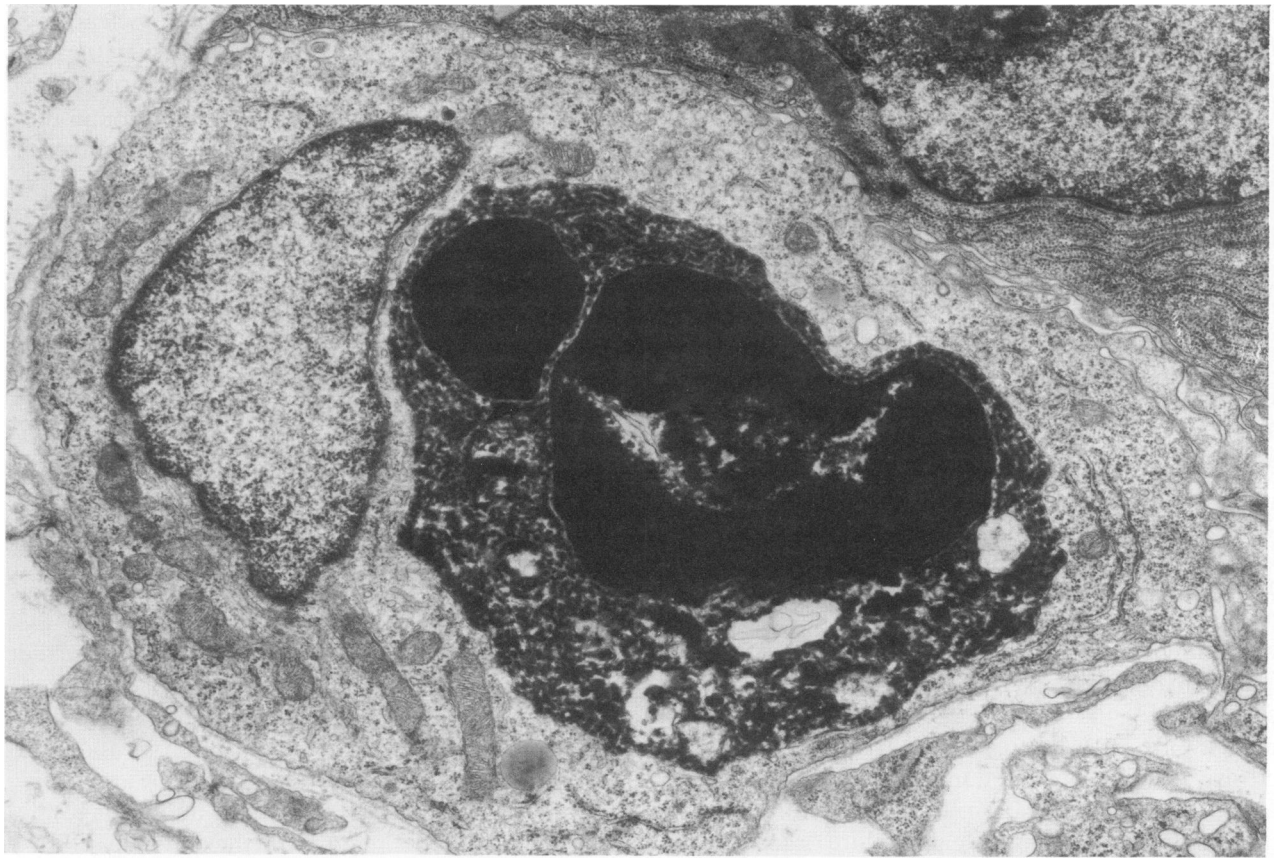
In the distal pancreas after ligation, centroacinar cells and cells of the intercalated ducts increased in size; occasional cells of these types were seen undergoing mitosis on Day 2, and large numbers on Days 3 and 4. The duct cell proliferation led to partial replacement of acinar cells by duct epithelial cells and, by 5 days, when most acinar cells had been deleted, to the formation of the characteristic lesion of lobular atrophy described in the introduction. Over a period of weeks and months, occasional duct epithelial cells were observed to undergo mitosis. Moreover, small numbers of duct cells, some showing partial acinar cell differentiation, continued to be deleted by apoptosis.

Discussion

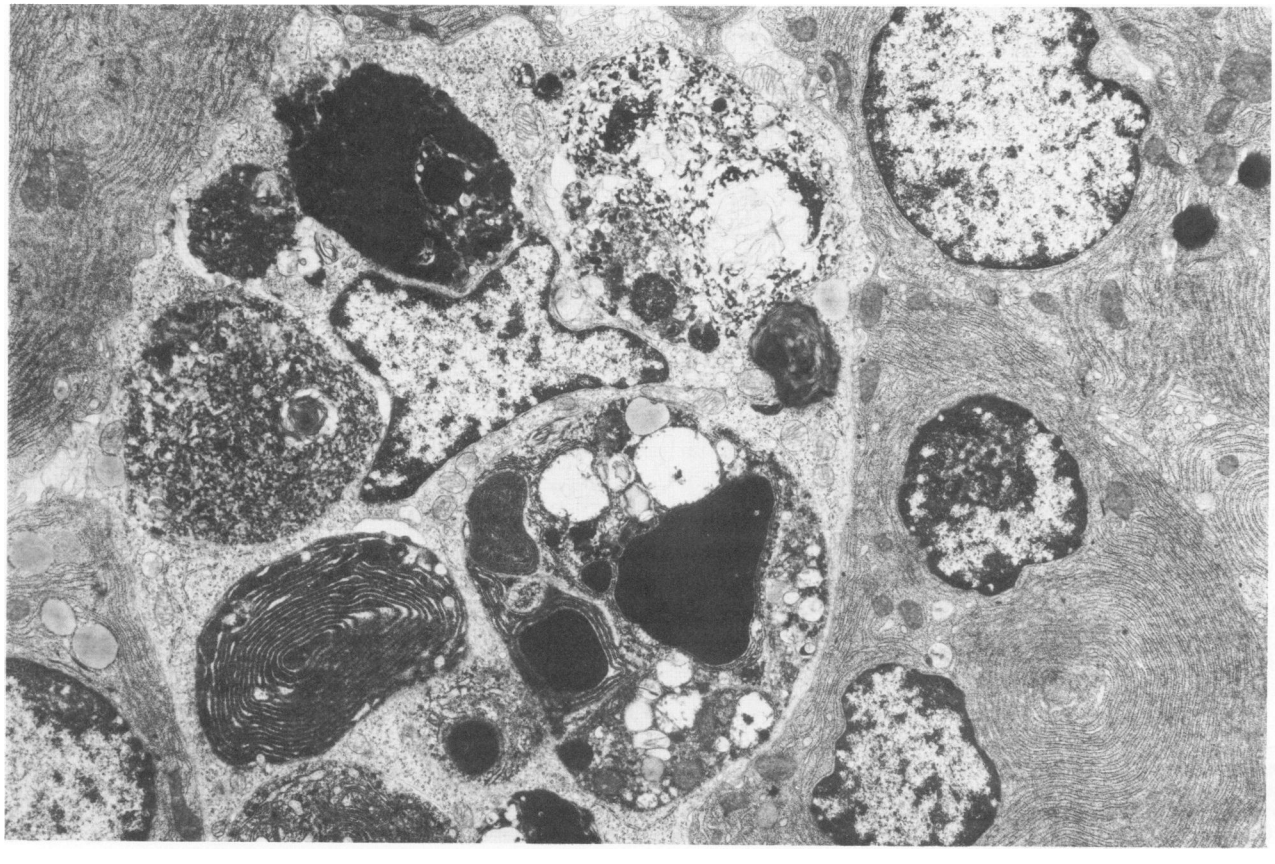
Acinar cells, which constitute 82% by volume of the mammalian pancreas,[21] disappeared from the distal part of the organ between 1 and 5 days after ligation. During this period, apoptosis was the only form of acinar cell death detected by light and electron microscopy, and the peak incidence of apoptosis at 3 days corresponded with the time of most rapid reduction in acinar cell numbers. Acinar cell death occurring spontaneously in the normal pancreas also took the form of apoptosis.

Apoptosis has been identified as the form of cell death responsible for normal involution and pathologic atrophy of tissues occurring under several different circumstances.[11-14] For example, it accounts for cell deletion during normal embryogenesis and metamorphosis, and it is involved in the shrinkage of adult endocrine-dependent tissues that follows withdrawal of trophic hormones. It is the mode of cell death in atrophy produced by mild ischemia, and it contributes to cell loss in neoplasms induced by radiation and cytotoxic drugs. The distinctive sequence of morphologic events in apoptosis comprises compaction and

Figure 11 — Activated intraepithelial mononuclear phagocyte in the distal pancreas 72 hours after ligation. Note the enlarged nucleus, decreased heterochromatin, and abundant cytoplasm. A phagocytosed apoptotic body shows partly degraded nuclear fragments and RER. (×14,300) **Figure 12** — Intraepithelial mononuclear phagocyte in the distal pancreas 60 hours after ligation. Phagocytosed apoptotic bodies show different degrees of enzymatic degradation. (×7800)



11



12

margination of chromatin against the nuclear membrane, condensation of cytoplasm with crowding of well-preserved organelles, cell budding to form membrane-bounded apoptotic bodies, and phagocytosis of the bodies by both epithelial cells and mononuclear phagocytes.[11-14] These features were all observed in the pancreatic exocrine tissue after duct obstruction. In addition, acinar cells dying by apoptosis showed rearrangement of RER into a series of circumscribed whorls prior to cell fragmentation, which appeared to occur between areas of segregated RER. The abundant RER present in acinar cells may allow visualization of cytoplasmic reorganization, which presumably always accompanies the cellular budding in apoptosis.

The morphologic features of apoptosis are quite distinct from those of necrosis,[22,23] which is characterized by severe swelling of mitochondria, the appearance of flocculent and granular densities in their matrix, and rupture of organelle and plasma membranes.[24] The two processes also differ biochemically. In apoptosis, there is selective cleavage of nuclear DNA at the linker regions between nucleosomes,[25-28] whereas this is not observed in necrosis.[28] Necrosis was seen in a few acinar cells in the first 24 hours after ligation. It may result from local trauma during the operative procedure or leakage of enzymes.

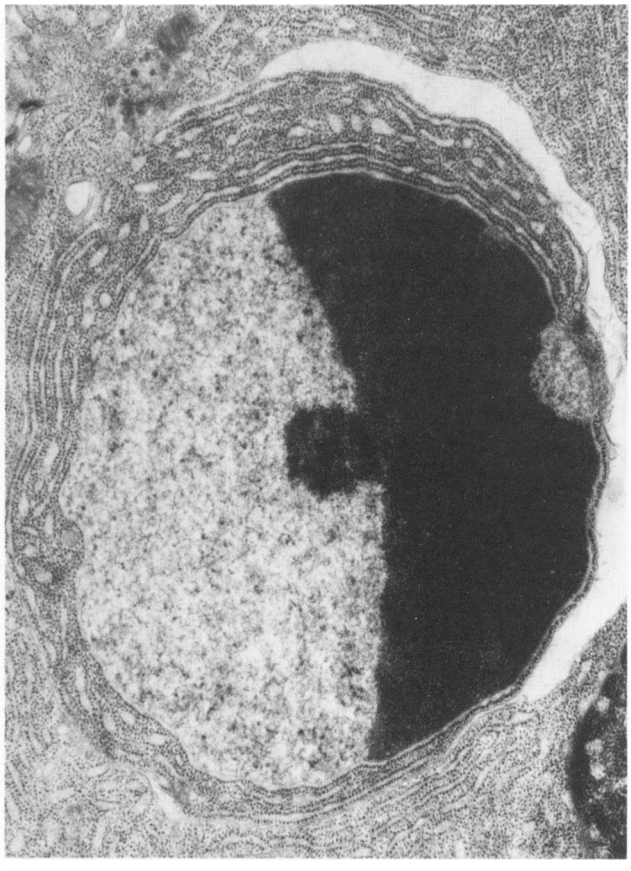
Despite rapid atrophy of the ligated pancreas, the cell death remained relatively inconspicuous. This is a consequence of the asynchrony of onset of apoptosis in different cells, the small size of many apoptotic bodies, and the rapidity with which they are removed.[11-14] The morphologically distinctive early stages of apoptosis progress rapidly and are relatively infrequently seen during ultrastructural studies, whereas the enzymatic degradation of phagocytosed apoptotic bodies takes much longer, leading to a relative abundance of phagolysosomes in tissues in which cell death by apoptosis is occurring.[11-14] Although most of the apoptotic bodies were disposed of by macrophages, some appeared to be taken up by epithelial cells. However, differentiation of phagocytosed apoptotic bodies from autophagic vacuoles in acinar cells was difficult. Small vacuoles containing RER, mitochondria, and zymogen granules, present in large numbers in each acinar cell during the first 24 hours after ligation, were probably autophagic in nature. Phagosomes in acinar cells at later times were mostly larger in size and sometimes contained nuclear fragments with the characteristic chromatin pattern of apoptosis, suggesting they were phagocytosed apoptotic bodies. Although ingestion of apoptotic bodies by epithelial cells has been established in a number of tissues,[11-14] pancreatic acinar cells are often binucleate,[18] and it is possible that nuclear fragments in acinar cell phagolysosomes might derive from one of the cell's own nuclei.

The initiating stimulus for the pancreatic acinar cell apoptosis induced by duct ligation is unknown. The time course was similar to that in involution of the rat adrenal[29] and ventral prostate[30] after reduction in circulating trophic hormone levels. In the pancreas, regression of diet-induced hyperplasia, which is effected by apoptosis,[31] is believed to be due to a fall in plasma cholecystokinin. After duct obstruction, however, plasma cholecystokinin levels are increased.[32] Moreover, the acinar cell loss is not generalized, implicating factors in the obstructed portion of the gland. Acutely decreased perfusion of the obstructed rat pancreas attributed to compression of blood vessels by edema fluid has been demonstrated.[33] Decreased supply of nutrients to cell cultures[34] and mild ischemia of the liver[35] enhance cell loss by apoptosis. The role of ischemia in causing the apoptosis in the obstructed pancreas requires further investigation.

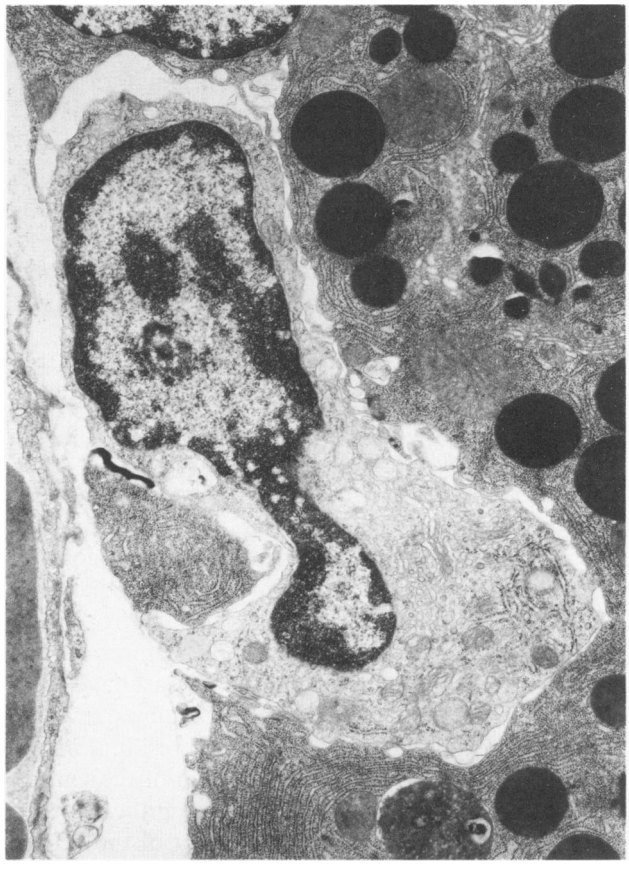
Mononuclear phagocytes were identified within the epithelium of the control rat pancreas. They varied morphologically from small cells resembling lymphocytes to larger macrophages with indented nuclei and abundant cytoplasm containing prominent Golgi complexes and occasional residual bodies. Despite their varied morphologic features, they all behaved as macrophages in the obstructed pancreas. Similar intraepithelial mononuclear phagocytes have been described in the ventral rat prostate,[30,36] the human and rat mammary gland,[37,38] and the human and hamster endometrium.[39,40] In most of these situations, phagocytosis of apoptotic bodies derived from epithelial cells has drawn attention to their presence.[30,37,39,40]

The intraepithelial mononuclear phagocytes in the pancreas became activated[41] after duct ligation. They grew in size, developed increased numbers of organelles, and showed enhanced phagocytic activity. By 72 hours, many closely resembled adjacent epithelial cells in their size and nuclear morphologic features. Nevertheless, they could be distinguished from the latter by their basal location and their lack of special-

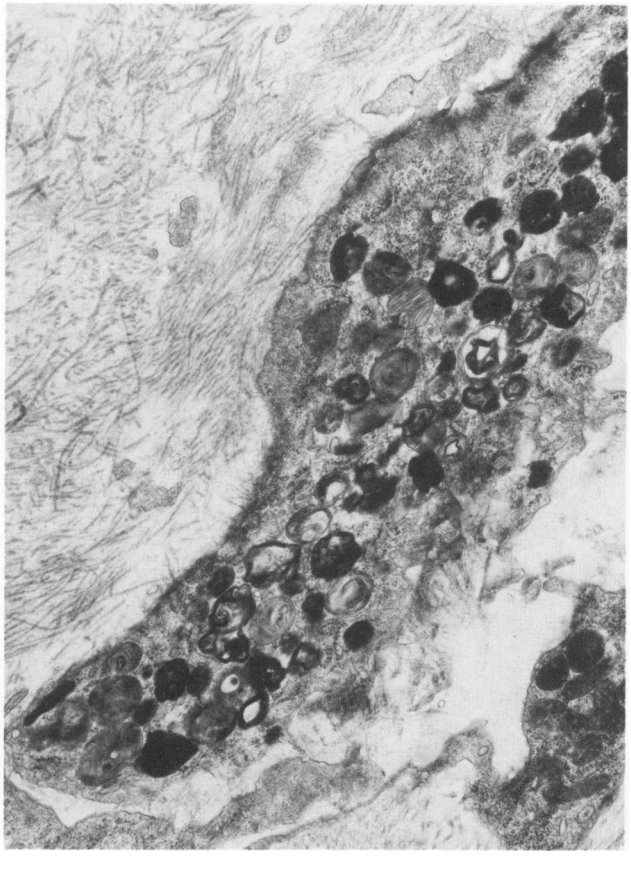
Figure 13—Membrane-bounded apoptotic body of acinar cell origin in normal pancreas. The ultrastructural appearances are essentially identical to those observed in the obstructed pancreas. (×15,800) **Figure 14**—Enlarged intraepithelial mononuclear phagocyte in the distal pancreas 24 hours after ligation. (×10,250) **Figure 15**—Residual body laden mononuclear phagocyte in interstitium of the distal pancreas 6 months after ligation. (×14,300) **Figure 16**—Intraepithelial mononuclear phagocyte in mitosis in the distal pancreas 14 days after ligation. It contains degraded phagocytosed cellular material. (×7100)



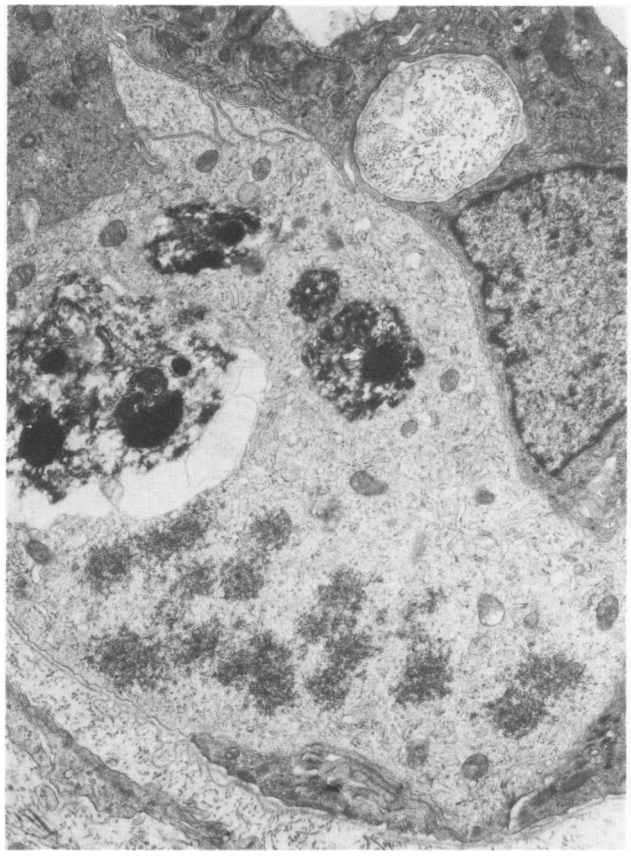
13



14



15



16

ized junctions. From 24 hours, phagosomes containing organelles foreign to the macrophage and identifiable as of acinar cell origin were found within them; and during the period of maximum acinar cell loss (60–72 hours), the macrophages often contained several ingested apoptotic bodies that showed evidence of enzymatic degradation of varying degree. Apoptotic bodies were clearly preferentially phagocytosed and digested by the intraepithelial macrophages. They probably also play a role in the phagocytosis of dying acinar cells in the normal pancreas.

The number of intraepithelial macrophages increased during the phase of rapid involution of the ligated pancreas, a result of both mitotic division and migration of blood monocytes into the epithelium. Similar increases in intraepithelial macrophages have been recorded in involuting rat breast[42] and prostate,[36] and migration of mononuclear phagocytes through the epithelial basement membrane has also been described and illustrated during atrophy of human and hamster endometrium.[39,40] Moreover, intraepithelial macrophages in human breast have been shown to bear cell membrane markers of marrow-derived cells.[43] The later appearance of residual body-laden macrophages in the interstitium of the ligated pancreas as their numbers within the epithelium decreased indicated that they moved out of the acinar walls.

Previous studies showing duct cell proliferation after pancreatic ligation[1,3,44] were confirmed. It is concluded that orderly remodeling of pancreatic exocrine tissue during atrophy is effected by rapid deletion of acinar cells by apoptosis and simultaneous proliferation of centroacinar and intercalated duct epithelial cells. As in other examples of tissue shrinkage mediated by apoptosis, the cell deletion occurs without disruption of the basement membrane.[11-14,29,30,35,45] In the longer term, the duct cell proliferation continued at a reduced rate, and some of the duct cells continually developed ultrastructural features indicating acinar cell differentiation; as they differentiated, they died by apoptosis. This latter sequence will be reported in detail elsewhere.

References

1. Boquist L, Edström C: Ultrastructure of pancreatic acinar and islet parenchyma in rats at various intervals after duct ligation. Virchows Arch [A] 1970, 349:69–79
2. Churg A, Richter WR: Early changes in the exocrine pancreas of the dog and rat after ligation of the pancreatic duct: A light and electron microscopic study. Am J Pathol 1971, 63:521–534
3. Pound AW, Walker NI: Involution of the pancreas after ligation of the pancreatic ducts: I. A histological study. Br J Exp Pathol 1981, 62:547–558
4. Zeligs JD, Janoff A, Dumont AE: The course and nature of acinar cell death following pancreatic ligation in the guinea pig. Am J Pathol 1975, 80:203–226
5. Longnecker DS: Pathology and pathogenesis of diseases of the pancreas. Am J Pathol 1982, 107:103–121
6. Bockman DE, Black O, Mills LR, Webster PD: Origin of tubular complexes during induction of pancreatic adenocarcinoma by 7, 12 dimethylbenz(a)anthracene. Am J Pathol 1978, 90:645–658
7. Bockman DE: Cells of origin of pancreatic cancer: Experimental tumours related to human pancreas. Cancer 1981, 47:1528–1534
8. Walters MN-I, Archer JM, Papadimitriou JM: Pancreatic duct obstruction: Ultrastructural morphology of the acute phase. Pathology 1969, 1:95–104
9. Edlund Y, Ekholm R, Zelander T: The morphological basis of acute pancreatic oedema: A light and electron microscopical study on rats. Acta Chir Scand 1962, 123:389–395
10. Zelander T, Ekholm R, Edlund Y: The ultrastructure of the rat exocrine pancreas after experimentally occluded outflow. J Ultrastruct Res 1964, 10:89–102
11. Kerr JFR, Wyllie AH, Currie AR: Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 1972, 26:239–257
12. Wyllie AH, Kerr JFR, Currie AR: Cell death: The significance of apoptosis. Int Rev Cytol 1980, 68:251–306
13. Wyllie AH: Cell Death: A new classification separating apoptosis from necrosis, Cell Death in Biology and Pathology. Edited by ID Bowen, RA Lockshin. London, Chapman and Hall 1981, 9–34
14. Kerr JFR, Searle J, Harmon BV, Bishop CJ: Apoptosis, Perspectives on Mammalian Cell Death. Edited by CS Potten. Oxford, Oxford University Press (In press)
15. Hultquist GT, Jönsson LE: Ligation of the pancreatic duct in rats. Acta Soc Med Upsal 1965, 70:82–88
16. Richards C, Fitzgerald PJ, Carol B, Rosenstock L, Lipkin L: Segmental division of the rat pancreas for experimental procedures. Lab Invest 1964, 13:1303–1321
17. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy (Abstr). J Cell Biol 1965, 27:137A–138A
18. Herman L, Sato T, Fitzgerald PJ: The pancreas, Electron Microscopic Anatomy. Edited by SM Kurtz. New York, Academic Press, 1964, pp 59–95
19. Ekholm R, Zelander T, Edlund Y: The ultrastructural organization of the rat exocrine pancreas: I. Acinar cells. J Ultrastruct Res 1962, 7:61–72
20. Ekholm R, Zelander T, Edlund Y: The ultrastructural organization of the rat exocrine pancreas: II. Centroacinar cells, intercalary and intralobular ducts. J Ultrastruct Res 1962, 7:73–83
21. Bolender RP: Stereological analysis of the guinea pig pancreas: I. Analytical model and quantitative description of nonstimulated pancreatic exocrine cells. J Cell Biol 1974, 61:269–287
22. Searle J, Lawson TA, Abbott PJ, Harmon B, Kerr JFR: An electron-microscope study of the mode of cell death induced by cancer-chemotherapeutic agents in populations of proliferating normal and neoplastic cells. J Pathol 1975, 116:129–138
23. Searle J, Kerr JFR, Bishop CJ: Necrosis and apoptosis: Distinct modes of cell death with fundamentally different significance. Pathol Annu 1982, 17(2):229–259
24. Trump BF, Berezesky IK, Osornio-Vargus AR: Cell death and the disease process: The role of calcium,[13] pp 209–242
25. Wyllie AH: Glucocorticoid-induced thymocyte apo-

ptosis is associated with endogenous endonuclease activation. Nature 1980, 284:555–556
26. Wyllie AH, Morris RG, Smith AL, Dunlop D: Chromatin cleavage in apoptosis: Association with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol 1984, 142:67–77
27. Cohen JJ, Duke RC: Glucocorticoid activation of a calcium dependent endonuclease in thymocyte nuclei leads to cell death. J Immunol 1984, 132:38–42
28. Duke RC, Chervenak R, Cohen JJ: Endogenous endonuclease induced DNA fragmentation: An early event in cell mediated cytolysis. Proc Natl Acad Sci USA 1983, 80:6361–6365
29. Wyllie AH, Kerr JFR, Macaskell IAM, Currie AR: Adrenocortical cell deletion: The role of ACTH. J Pathol 1973, 3:85–94
30. Kerr JFR, Searle J: Deletion of cells by apoptosis during castration induced involution of the rat prostate. Virchows Arch [Cell Pathol] 1973, 13:87–102
31. Oates PS, Morgan RGH, Light AM: Cell death (apoptosis) during pancreatic involution following raw soya flour feeding in the rat. Am J Physiol 1986, 250:G9–G14
32. Inoue K, Yazigi R, Watson LC, Gourley WK, Rayford PL, Thompson JC: Increased release of cholecystokinin after pancreatic duct ligation. Surgery 1982, 91:467–473
33. Edström C, Hassler O: Functional capacity, alloxan sensitivity, and vascular supply of pancreatic islet parenchyma during the first week after duct ligation. Acta Soc Med Upsal 1971, 76:211–222
34. Sheridan JW, Bishop CJ, Simmons RJ: Biophysical and morphological correlates of kinetic change and death in a starved human melanoma cell line. J Cell Sci 1981, 49:119–137
35. Kerr JFR: Shrinkage necrosis: A distinct mode of cellular death. J Pathol 1971, 105:13–20
36. Helminen HJ, Ericsson JLE: Ultrastructural studies on prostatic involution in the rat: Evidence for focal irreversible damage to epithelium and heterophagic digestion in macrophages. J Ultrastruct Res 1972, 39:443–455
37. Ferguson DJP, Anderson TJ: Ultrastructural observations on cell death by apoptosis in the "resting" human breast. Virchows Arch [A] 1981, 393:193–203
38. Helminen HJ, Ericsson JLE: Studies on mammary gland involution: I. On the ultrastructure of the lactating mammary gland. J Ultrastruct Res 1968, 25:193–213
39. Hopwood D, Levison DA: Atrophy and apoptosis in the cyclical human endometrium. J Pathol 1976, 119:159–166
40. Sandow BA, West NB, Norman RL, Brenner RM: Hormonal control of apoptosis in hamster uterine luminal epithelium. Am J Anat 1979, 156:15–36
41. Adams DO: The structure of mononuclear phagocytes differentiating in vivo: I. Sequential fine and histologic studies of the effects of bacillus Calmette–Guerin. Am J Pathol 1974, 76:17–48
42. Helminen HJ, Ericsson JLE: Studies on mammary gland involution: II. Ultrastructural evidence for auto and heterophagocytosis. J Ultrastruct Res 1968, 25:214–227
43. Ferguson DJP: Intraepithelial lymphocytes and macrophages in the normal breast. Virchows Arch [A] 1985, 407:369–378
44. Walker NI, Pound AW: An autoradiographic study of the cell proliferation during involution of the rat pancreas. J Pathol 1983, 139:407–418
45. Weedon D, Strutton G: Apoptosis as the mechanism of the involution of hair follicles in catagen transformation. Acta Derm Venereol (Stockh) 1981, 61:335–339

Acknowledgments

The author thanks Glenda Gobé for technical assistance and Professor J. F. R. Kerr for comments on the manuscript.